

Risk Assessment for the Harmful Effects of UVB Radiation on the Immunological Resistance to Infectious Diseases

Wim Goettsch,¹ Johan Garssen,¹ Wout Slob,¹ Frank R. de Gruijl,² and Henk Van Loveren¹

¹National Institute of Public Health and Environmental Protection, Bilthoven, The Netherlands; ²Department of Dermatology, University of Utrecht, Utrecht, The Netherlands

Risk assessment comprises four steps: hazard identification, dose-response assessment, exposure assessment, and risk characterization. In this study, the effects of increased ultraviolet B (UVB, 280–315 nm) radiation on immune functions and the immunological resistance to infectious diseases in rats were analyzed according to this strategy. In a parallelogram approach, nonthreshold mathematical methods were used to estimate the risk for the human population after increased exposure to UVB radiation. These data demonstrate, using a worst-case strategy (sensitive individuals, no adaptation), that exposure for approximately 90 min (local noon) at 40°N in July might lead to 50% suppression of specific T-cell mediated responses to *Listeria monocytogenes* in humans who were not preexposed to UVB (i.e., not adapted). Additionally, a 5% decrease in the thickness of the ozone layer might shorten this exposure time by approximately 2.5%. These data demonstrate that UVB radiation, at doses relevant to outdoor exposure, may affect the specific cellular immune response to *Listeria* bacteria in humans. Whether this will also lead to a lowered resistance (i.e., increased pathogenic load) in humans is not known, although it was demonstrated that UVB-induced immunosuppression in rats was sufficient to increase the pathogenic load. Epidemiology studies are needed to validate and improve estimates for the potential effects of increased UVB exposure on infectious diseases in humans. **Key words:** immunosuppression, immune system, *Listeria*, risk assessment, UVB radiation. *Environ Health Perspect* 106: 71–77(1998). [Online 21 January 1998]

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Experimental data on the effects of ultraviolet B (UVB) radiation on infectious diseases, easily obtained in laboratory animals, cannot be acquired using humans for ethical reasons. Epidemiological data are not available until now. Even the effect of sunlight on fever blisters, a well-known skin infection resulting from the Herpes simplex virus and associated with sun exposure, has not been analyzed in quantitative terms. One of the major problems of epidemiology is the large number of confounding factors. People exposed to increased UVB levels (during sun holidays) are also affected by other potential immunomodulatory factors such as stress, diet, temperature, etc. A possible alternative is extrapolation of animal data to humans with respect to the effects on infectious diseases. These extrapolated data may be used as a basis for risk and effect assessment of immunotoxicological agents such as ultraviolet light.

Risk assessment is a process of analyzing relevant biological, dose-response, and exposure data for a particular agent in an attempt to establish qualitative and quantitative estimates of adverse effects on human health (1). As defined by the frequently cited National Academy of Science Report (2), risk assessment comprises four steps: hazard identification, dose-response assessment, exposure assessment, and risk characterization. This general framework covers the process of assessing risk of cancer as well as

noncancer endpoints, including decreased resistance to infections. The present study is not aimed at the determination or calculation of the maximal tolerable dose of UV radiation humans are advised or allowed to be exposed to. This study only serves as an estimation of the effect of exposure to increased levels of UVB with respect to the immune system and related resistance to infections in comparison to humans that are not exposed to increased UVB levels due to ozone depletion.

The first step, hazard identification, involves a largely qualitative evaluation of available human and animal data to determine whether a chemical or physical agent is a potential hazard. Consideration is given to the dose, route, and duration of exposure in the test species. Any possible change in immune function, due to the agent studied, indicates that this agent is a potential hazard. Many studies indicate that suberythral doses of ultraviolet light can affect the immune system in rodents as well as in humans. The major effect of UV exposure is suppression of the cellular immune system and of natural killer cell (NK) function, which is found in humans as well as in rodents. In rodent studies it has been demonstrated that UV-induced immunomodulation can lead to a lowered resistance to certain infections and tumors (3–17).

Following hazard identification, dose-response studies (step 2 of risk assessment)

give quantitative information, which is the basis for obtaining the no-observed adverse effect level (NOAEL) and the lowest adverse effect level (LOAEL), which are threshold values. This information is also necessary to determine the effective dose, which induces a certain level of suppression of an immune function, e.g., 50% suppression (ED50), for which it is assumed that there is no threshold dose value. The ED50, the dose that inhibits the response by 50%, can be calculated using linear or nonlinear regression models that quantify the relationship between dose and effect by a mathematical expression. In studies concerning the immunosuppressive effects of UVB, the ED50 is frequently used (3). For many immune parameters, NOAEL, LOAEL, and ED50 levels are still not available. In most studies only one or two different UV doses were tested; thus, dose-response analyses are only available in a few cases. Once these entities are determined, specific factors can be applied to allow for various phenomena such as intraspecies or interspecies variability, irreversible effects, and duration of exposure. The use of these specific factors, however, should be flexible and incorporate any relevant available data on the mechanism of action of the particular agent. One approach that has been used is the application of an uncertainty factor to the NOAEL established in the most sensitive animal species tested. This uncertainty factor is usually composed of a 10-fold factor to account for interspecies differences and a 10-fold factor for the intraspecies factor. If a NOAEL is not available, an additional 10-fold factor may be applied to the LOAEL. These factors are all used to calculate or estimate the maximal tolerable dose, i.e., the safe dose, for human beings. Literature data on the differences in susceptibility for UVB-induced degenerative effects indicate that a 10-fold factor for interspecies extrapolation overestimates the actual difference (4–8).

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The third step in risk assessment, exposure assessment, often involves field measurements on the agent and other estimates relating to human exposure such as composition and size of population, biological and clinical effects, and types, magnitude, frequencies, and duration of exposure to the agent.

Risk characterization (step 4) is the integration of the previous three processes; it provides an estimated incidence of the adverse effects in populations and the potential health effects. As part of risk characterization, the strength and weaknesses in each component of the assessment are considered including assumptions, scientific judgments, and to the greatest extent possible, estimate of uncertainties.

In this paper, we describe an attempt to assess the effects of increased UVB radiation, due to changes in the ozone layer, on the immunological resistance to infectious diseases. For this risk assessment, a worst-case approach was employed, i.e., adaptation to UVB was not taken into account and non-threshold (ED50) methods were used. For the most part, data on the effects of UVB on the immune system are from our laboratory.

Hazard identification. Immunological studies showing the effects of UVB exposure on the immune system have been described and reviewed extensively. Hurks et al. (18) reviewed the general aspects of UV radiation on the immune system. They showed that suberythral doses [0.25 and 0.50 of the minimal erythral dose (MED) for the rat] of UVB radiation daily for 7 consecutive days could induce severe suppression of NK activity and mixed lymphocyte responsiveness (MLR), which is a valuable parameter for the cellular (T-cell dependent) immune system of splenocytes

in rats (9,10). In addition, several *in vitro* (cell suspensions), *in situ* (skin sheets), and *in vivo* experiments indicated that UVB exposure impaired several immune functions such as T-cell function (MLR responses) and the alloreactive capacity of (epi)dermal cells [mixed skin lymphocyte response (MSLR)] in mice, rats, and humans in a comparable fashion (9,11,12). The MSLR is a valid parameter for the initiation phase of the immune response (i.e., antigen presentation).

In a *Listeria monocytogenes* host resistance model in the rat a similar UVB exposure protocol (i.e., 0.5 MED daily for 7 consecutive days) resulted in an eightfold increase in the number of bacteria in the spleen, which is a target organ for this bacterium after intravenous infection; this result was found 4 days after infection (13). Delayed type hypersensitivity responses and specific lymphocyte proliferation assays, both parameters for T-cell mediated immunity to *Listeria*, were inhibited by these UVB exposure protocols, indicating that UVB exposure induces suppression of the specific T cell-mediated immune response to *Listeria*, leading to delayed clearance of bacteria from the spleen. Infection of rats with the parasite *Trichinella spiralis* was also affected by UVB exposure (14). The 7-day UVB exposure protocol, starting 7 days after infection, induced increased numbers of larvae in the muscle tissue of the infected rats, as detected by digestion and histological methods. In addition, cellular immune responses to *T. spiralis* were inhibited by UVB exposure (15). Cellular immune responses to herpes simplex, type 1 (skin-associated) and rat cytomegalovirus (nonskin-associated) were also inhibited by UVB exposure (16,17). In

the case of rat cytomegalovirus, this resulted in an increased amount of virus present in the salivary gland 26 days after infection. Detailed studies with the Herpes simplex I virus in the rat and in the mouse (17) indicated that the time point of exposure is very important for initiation of the immunosuppression.

From these studies it was concluded that low doses of UVB radiation, relevant to the outdoor situation, are able to impair the immune system. Thus, UVB radiation is a potential hazard to immunological functions and to the immunological resistance to infectious diseases in humans. Recent studies indicate that if animals were exposed for longer periods to UVB, the immunosuppressive effects were less pronounced. This indicates that adaptation processes may play a role and ultimately may lead to less severe effects. In this paper we describe how we characterized the effect of immunosuppression by ultraviolet irradiation in humans; adaptation processes were not taken into account in this study.

Methods

Dose-response assessment. In order to perform dose-response assessments, various models were developed to test the immunotoxic effects of UVB radiation on the immune response and, more precisely, on the immunological resistance to infectious diseases. Dose-response assessment was applied using linear and nonlinear regression methods to evaluate the relationship between dose and effect and to calculate an ED50 value. Data obtained from both methods of analysis were used to compose a factor to account for interspecies variation (IEV; between different species, i.e. rats and

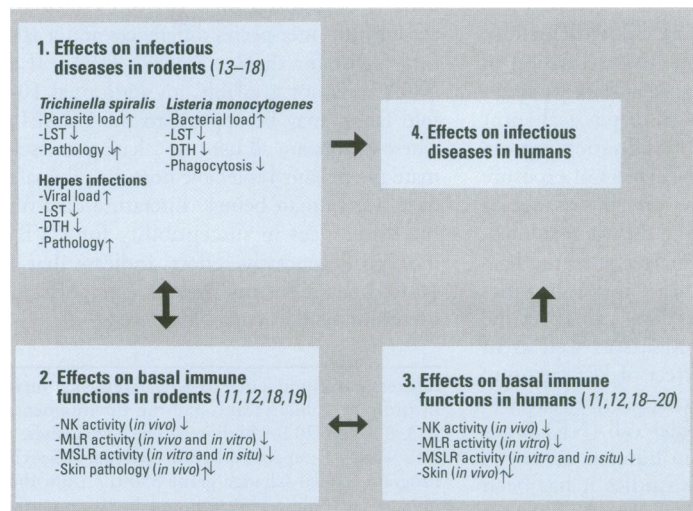


Figure 1. Parallelogram for the effects of ultraviolet B radiation on the resistance to infectious diseases. Abbreviations: LST, lymphocyte stimulation test; DTH, delayed type hypersensitivity; NK, natural killer cell; MLR, mixed lymphocyte responsiveness; MSLR, mixed skin lymphocyte response.

Table 1. Analysis of ultraviolet B radiation doses (kJ/m²) using FS40 lamps necessary to obtain the 50% immunosuppression level (ED50) for the parameters presented

Parameters	ED50
Effects on immunological resistance to infectious diseases in rodents	
LST <i>Listeria monocytogenes</i> ^a	6.8
Effects on immune functions in rodents	
MLR (<i>in vivo</i>) ^a	1.2
NK (<i>in vivo</i>) ^a	5.9
MSLR (<i>in vitro</i>)	0.04/0.08 ^b
MSLR (<i>in situ</i>)	0.7/1.1 ^b
MLR (<i>in vitro</i>)	0.15/0.05 ^b
Effects on immune functions in humans	
MSLR (<i>in vitro</i>)	0.2
MSLR (<i>in situ</i>)	4.2
MLR (<i>in vitro</i>)	0.13

Abbreviations: LST, lymphocyte stimulation test; NK, natural killer cell; MLR, mixed lymphocyte responsiveness; MSLR, mixed skin lymphocyte response.

^aThese data were used to compare effects of UVB radiation on immunological resistance to infectious diseases and effects on immune functions in rodents. All other data were used to compare effects on immune functions in rodents and effects on immune functions in humans.

^bThe first value is for mouse and the second is for rat.

humans). Some models could also be used to compose a factor for intraspecies variation (IAV; between different human subjects). Figure 1 illustrates the paradigm used for human dose–response assessment by extrapolation from animal data (13–20).

Effects of UVB on the resistance to infectious diseases in rodents. A dose–response curve of the effect of UVB exposure on the immunological resistance to *L. monocytogenes* (13) indicated a dose-dependent decrease of the specific lymphocyte proliferation (*ex vivo/in vitro*) induced by coculture of lymphocytes with heat-killed *Listeria* bacteria [lymphocyte stimulation test (LST)]. The proliferation was measured using incorporation of ^3H -thymidine. Using log-transformed LST data, a dose–response curve and an $\text{ED}_{50_{\text{Lis,LST}}}$ (UV dose necessary to impair the LST to *Listeria* by 50%) was calculated (Table 1).

Effects of UVB on immune functions in rodents. *In vivo* UVB radiation dose-dependently suppressed NK and MLR activity of splenocytes or blood cells, and ED_{50} values were calculated (Table 1) (9). ED_{50} values were also estimated from data on the effects of acute *in vitro* UVB exposure (11) on log MLR responses (Table 1). The effects of *in vitro* (cell suspensions) and *in situ* (skin pieces) exposure on the allogenic response measured in the MSLR indicated that the allogenic capacity of epidermal cells (model for antigen presentation) from rats and mice was dose-dependently suppressed (12).

Effects of UVB radiation on immune functions in humans. *In vitro* UVB exposure of blood cells and *in vitro* and *in situ* UVB exposure of epidermal cells indicated a dose-dependent decrease of MLR (11) and MSLR (12) (Table 1). For the estimation of the intraspecies variation (IAV) in the human population, linear regression was performed with both the intercept and the slope considered as normally distributed random variables, representing interindividual variation. This model was fitted to the human data using an approach for the analysis of generalized linear mixed models as published by Engel and Keen in 1994 (21). The variance, indicated in the formula as standard deviation (SD) of the slope (b), was estimated and represents interindividual variation (susceptibility). Thus, the susceptibility of 5% of the population is larger than

$$b_{95\%} > b + z \times \text{SD}, \quad (1)$$

where z is the standardized normal deviate = 1.645.

We define the IAV as

$$\text{ED}_{50_{95\%}}/\text{ED}_{50_{\text{mean}}} = b_{\text{mean}}/b_{95\%}. \quad (2)$$

IAV for the $\text{MSLR}_{\text{human, in situ}}$ (mixed skin lymphocyte response after exposure of human epidermal skin sheets) was estimated at a factor 0.5 (average subject relative to sensitive subject) (12).

Relationships were assessed between the effects of UVB exposure on immune functions and the resistance to infectious diseases in rodents. ED_{50} values of the host resistance and the immune parameters indicated that the MLR and NK were somewhat more susceptible for UVB than the LST to *L. monocytogenes* (Table 1). Nevertheless, the MLR and NK, as well as the LST to *Listeria* were significantly suppressed after comparable cumulative doses of 1–10 kJ/m^2 UVB radiation. Doses in the same range were able to impair the overall resistance to *L. monocytogenes*, *T. spiralis*, and cytomegalovirus in the rat (13,14,16).

Using a similar UVB exposure protocol in rodents and humans, it was possible to compare the dose–response relationship. The effects of *in vitro* UVB exposure on the MLR activity of splenocytes did not indicate significant differences (Table 1). The effects of *in vitro* and *in situ* UVB exposure on alloreactive capacity of epidermal cells [MSLR; i.e., a parameter for the initiation of immune responses (antigen presentation)] indicated that the human cells were less susceptible for UVB radiation. ED_{50} values of humans, mice, and rats indicated a four- to sixfold factor in susceptibility for *in vitro* and *in situ* UVB exposure between rats and mice on the one hand and humans on the other (Table 1).

Calculation/estimation of the ED_{50} value for humans for the effects of UVB exposure on the resistance to infectious diseases. We used the ED_{50} of the specific T-cell response to *Listeria* because the data permitted a reliable dose–response curve (13). In addition, a UVB-induced suppression of specific T-cell responses to *Listeria* may lead to an increased load of bacteria in the spleen. The dose necessary to inhibit the specific lymphocyte response by 50% was sufficient to lead to a delayed clearance of the bacteria in the rat. Using all relevant and adequate data, the IEV between rats and humans was calculated as

$$\text{IEV} = \text{ED}_{50_{\text{human}}} / \text{ED}_{50_{\text{rat}}}$$

Analysis of all the immunological models used suggested some interspecies differences in susceptibility for UVB-induced effects on immune functions and the skin after *in vivo*, *in situ*, and *in vitro* UVB exposure. The IEV calculated had to satisfy certain conditions:

- The immunological model had to be well defined in order to accurately describe the dose–response dependency

for all the species tested. This could also lead to adequate ED_{50} values

- The model needed to be relevant for the mechanisms by which UVB radiation induces immunosuppression
- The UVB exposure of the different species had to be comparable to actual outdoor exposure (order of relevance: *in vivo* (exposure of volunteers) > *in situ* (exposure of skin sheets) > *in vitro* (exposure of skin cell suspensions or skin cell lines) > exposure of blood cells
- The UVB exposure protocol for all species had to be exactly the same (same sources, same dose range).

When all the conditions were examined in the immune function models (MLR and MSLR) (11), the inhibition of (allo)antigen presentation, as measured by MSLR, was more relevant to the mechanisms by which UVB radiation induces immunosuppression than the inhibition of the MLR activity of splenocytes or blood cells. Inhibition of functional activity of the Langerhans cells as measured in the MSLR seems to be a sensitive biomarker for effects of UVB on the skin (12) and also for systemic effects when those UVB-affected LC migrate to the lymph nodes (22). *In situ* UVB exposure (UVB exposure of skin pieces) was also more relevant for actual outdoor exposure than the *in vitro* UVB exposure of cell suspensions. Finally, the model describing the effects of *in situ* UVB exposure on the alloantigen presentation also satisfied the requirement for exact protocol duplication; all species were exposed to exactly the same source of UV under exactly the same conditions in the same laboratory.

The IEV was calculated by dividing the $\text{ED}_{50_{\text{MSLR, human}}}$ (4,176) by the $\text{ED}_{50_{\text{MSLR, rat}}}$ (1,085) leading to the IEV of 3.85. When the IEV factor was calculated using ED_{80} or ED_{20} values, the IEV factor was similar.

IAV was assessed in the MSLR assay at the value of 0.5.

Thus, $\text{ED}_{50_{\text{human, Lis, LST}}} =$

$$\text{ED}_{50_{\text{rat, Lis, LST}}} \times \text{IEV}_{\text{MSLR}} \times \text{IAV}_{\text{MSLR}}$$

$13.1 \text{ (kJ/m}^2\text{)} = 6.8 \text{ (kJ/m}^2\text{)} \times 3.85 \times 0.5$ (cumulative dose).

Exposure assessment. Exposure to UVB radiation can be divided into five categories:

- Exposure to sunlight, which can be divided into the normal exposure in the Netherlands and more excessive UVB exposure (when people are on summer holiday in, for example, southern European countries)
- Exposure to UVB by illumination, (e.g., by halogen desk lamps)

- Exposure to UVB radiation for cosmetic reasons
- Exposure to UVB radiation for therapy
- UVB exposure in the workplace.

In 1991 Slaper and Eggink (23) estimated the yearly exposure to UVB radiation of an average citizen in the Netherlands to be 112 minimal erythema doses (MEDs). The main part of this exposure is due to sunlight; however, there are special groups in the Dutch population for which the main part of exposure is due to artificial sources. In addition, excessive exposure to sunlight during summer holidays could lead to increases of 50–100 MEDs on a yearly basis (23,24).

It is clear that the UVB dose, expressed as MEDs, did not provide enough information on the immunosuppressive capacity of UVB radiation (9,10). Comparison of action spectra for erythema and suppression of contact hypersensitivity showed some differences. Suppression of the resistance to infections was mainly due to suppression of the activity of T-cell immunity (13–16). As a first step, it was postulated that the action spectrum for the suppression of the resistance to infectious diseases in rodents is similar to the action spectrum of the suppression of contact hypersensitivity (CHS) (25). CHS is a good test model for cellular immunity. De Fabo et al. (3) calculated biological effective irradiation for immunosuppression (BEI_{imm}) for several latitudes and time points in summer and winter. In this study, the spectrum of the sun [calculated from UV spectral irradiance incident on the earth's surface using the radiative transfer model summarized by Frederick and Lubin (26)] was multiplied by the action spectrum for suppression of CHS, and biological effective doses were calculated (Table 2). Each individual value of the action spectrum curve for immunosuppression as presented and calculated by De Fabo et al. (25) was taken into account in our exposure assessment, which is in contrast to a preliminary study published earlier in which less advanced computer programs were used (19).

Results

Risk characterization. The question that had to be raised was the relevance of an $ED50_{human,Lis,LST}$ of 13.1 kJ/m² UVB radiation (280–315 nm, FS40, North American Philips F40; Westinghouse, Bloomfield, NJ) to the actual exposure of people outdoors. Several studies showed that damaging effects of UV radiation were wavelength dependent, with UVC and short UVB responsible for the deleterious effects. UVC and short wavelengths of UVB are either hardly present or not present in normal sunlight. For

that reason, it was necessary to translate the dose of UVB, as mentioned in the dose–response assessment, into biologically relevant doses. However, for the calculation of a biologically effective dose concerning suppression of resistance to *Listeria*, a wavelength action spectrum for CHS in mice was used, as determined by de Fabo and Noonan (25). The spectrum of the FS40-lamp was multiplied by all data points of the action spectrum curve for CHS, as demonstrated schematically in Figure 2 by calculating a biological effective irradiance (BEI_{imm}) for every wavelength. Thus, even the shoulder (dip) in the action spectrum was taken into account. The area under the curve for FS40_{imm} was calculated, giving the total BEI_{imm} for the wavelengths between 280 and 315 nm (the influence of UVR >315 nm is negligible using FS40 lamps).

The value of this BEI_{imm} (280–315 nm) was 0.186 W/m². The integrated UVB irradiance of the FS40 curve, as measured by the Optronics 752-OL-PMT (Optronics, Orlando, FL) was 1.589 W/m² (uncorrected), so the BEI_{imm} was 11.7% of the total irradiance. It was calculated that the biological effective ED50 value for suppression of T-cell responses to *Listeria* was $13.1 \times 0.117 = 1.53$ kJ/m² (normalized to 270 nm).

The calculated biological effective irradiance for 50% immunosuppression (BEI_{imm}) of sun exposure at certain latitudes was related to immunosuppression found in our *Listeria* model, defined as $ED50_{bed,Lis,LST}$ (see Table 2). Dividing the $ED50_{bed,Lis,LST}$ by the BEI_{imm} , as calculated by De Fabo et al. (3), gave a first prediction on the duration of sun exposure (at a certain latitude, clear skies, local noon) necessary to induce 50% suppression of specific T-cell response to *Listeria*. For instance, it was predicted that 92 min exposure around noon, with clear skies in July at 40°N, would lead to 50% suppression of the specific T-cell

response to *Listeria*. In addition, assuming a 20% decrease of ozone, it was calculated that 82 min was enough to induce the same immunosuppression. As another example, in January at a latitude of 40°N, this immunosuppression might be induced after 350 min (19). When these data are compared to the UVB dose that induced increased load of *Listeria* in the rat spleen, it can be predicted that relevant UVB doses may lead to an impaired clearance of *Listeria* bacteria in humans that had not adapted to UVB exposure (13).

Discussion

Hazard estimation of the UVB-induced effects on the resistance to infectious diseases indicated that UVB radiation impaired cellular immune responses, leading to decreased resistance to different infectious agents in the rat (9–16).

Dose–response assessment of the effects of UVB radiation on the resistance to infectious agents and immune functions in mice, rats, and humans showed that there was a dose-dependent relationship between UVB exposure and several effects tested. The relationship between UVB exposure and suppression of immune functions such as the MLR and MSLR was quantified in linear and nonlinear regression models. For dose–response assessment, ED50 values were used instead of NOAEL and LOAEL values because ED50 values are more sensitive in detecting small differences induced by UVB exposure. For the ED50 calculation, all dose–response data, usually more than six dose groups, were used in a regression analyses, which is much more precise than the estimation of a NOAEL or LOAEL value; NOAELs and LOAELs are mostly dependent upon data from only two or three dose groups. In a linear regression model, all samples (*n*) are included in the determination of the standard error

Table 2. Predicted effects of ozone decreases on the biologically effective irradiance for suppression of the specific cellular immune response to *Listeria* bacteria (local noon, clear skies, southern Europe)

Latitude (month)	Ozone (dobson units) ³	Decrease in ozone ³ (%)	Biologically effective irradiance (W/m ²) ³	Increase in BEI_{imm} ³ (%)	RAF _{imm}	Calculated time (min) for 50% immunosuppression ^a
40°N (January)	335.6	0	0.073	0.0	–	350
	318.8	5	0.075	3.0	0.6	340
	302.0	10	0.078	6.3	0.63	327
	268.5	20	0.083	13.5	0.68	307
40°N (July)	307.9	0	0.278	0.0	–	92
	292.5	5	0.285	2.5	0.50	90
	277.1	10	0.292	5.3	0.53	87
	246.3	20	0.310	11.5	0.58	82

Abbreviations: BEI, biological effective irradiance; RAF, radiation amplification factor. Adapted from De Fabo et al. (3) and Garssen et al. (19) (with permission from *Photochemistry and Photobiology*).

^aLymphocyte proliferation in response to *Listeria* bacteria.

(standard deviation divided by the root square of n). This results in a smaller standard error than when two groups of the total population are compared. The significance of the difference between several groups is dependent on the size of the coefficient of the difference divided by the standard error; a larger coefficient indicates a stronger significant result. A second reason for using ED50s is that it is possible to compare the data with the action spectrum of De Fabo and Noonan (25), who calculated the action spectrum using ED50 values for contact hypersensitivity. Comparison of the effects of UVB exposure on immune functions and the immunological resistance to infectious diseases in rats indicate that there are no major differences in the sensitivity of those assays for UVB radiation. This result is important for the subsequent extrapolation of the effects of UVB exposure on immunological resistance in rats to humans. In viral infections, the activity of NK is important for the clearance of virus from the host. However, it has been demonstrated that the effects of immunotoxic compounds on immune parameters, such as NK activity, do not correlate to the effects of the same compounds on the resistance to cytomegalovirus (27). In such a case, the use of the parallelogram approach for the extrapolation of immunotoxic compounds is not complete because differences in susceptibility between rats and humans, measured by the NK assay, do not completely account for the actual differences in susceptibility between these

species for the resistance to virus infections. In addition, Noonan and Lewis (28) found that some immune parameters can be affected by UVB easily, without a detectable effect on the resistance to a pathogen such as *Schistosoma mansoni*. For that reason, it may be necessary to add other immune functions tests such as cytotoxic T-lymphocyte assays or lymphocyte stimulation tests to increase correlation between immune function and resistance.

Comparisons of the effects of UVB exposure on immune functions indicate that interspecies differences depend on the assay used, probably because each assay covers only a part of the immunological response. For example, the MSLR assay used for the calculation of the IEV primarily accounts for effects of UVB radiation on Langerhans cells in the skin and may also be relevant for systemic effects when the UVB-affected Langerhans cells migrate to the lymph nodes (22). However, the effects measured on MSLR may not always correlate to all effects of UVB exposure on immunological resistance to infectious diseases; UVB radiation can impair immune responses by different pathways, e.g., by impairing Langerhans cells and also by the induction of several cytokines that have local and systemic immunomodulatory effects (29). Therefore, the IEV used in this risk assessment may not cover all effects of UVB radiation on the immune system, and additional data are helpful. IEV and IAV values were calculated based on ED50 values for the different

species and individuals. If ED₂₀ or ED₈₀ values were used instead of ED50 values, similar interspecies and intraspecies factors were obtained. Studies on the effects of UVB exposure on the development of CHS in humans (4,30) and different strains of mice (31,32) showed a negligible interspecies variation, not exceeding a factor 1.5. This might indicate that the IEV we used is too high. The use of antigen-specific immunological assays, such as CHS and LST in combination with host-resistance models, may provide additional data on the IEV. For example, effects of UVB exposure on specific immune responses to herpes simplex, type 1 can be studied in herpes simplex, type 1-positive psoriasis patients and human volunteers and compared to similar studies in the rat. Another human infection that may produce additional data on the effects of UVB on the immunological resistance to infectious diseases and can be compared with studies in rodents, is human papilloma virus infection of the skin. There are indications that this infectious agent is also associated with certain forms of skin tumors (33,34).

Another problem in the analysis of the IEV is the presence of genetic differences. Studies by Streilein et al. (6) and Taylor et al. (35) provide evidence that differences in susceptibility for the effects of UVB radiation on CHS probably depends on several genetic factors. In mice it was demonstrated that UVB-induced immunosuppression is strain dependent and could be attributed to several genetic factors (36,37). Additional studies with human volunteers should be carried out using large groups of individuals to carefully estimate the influence of IAV. Another approach is to study extra-susceptible human individuals, such as skin cancer patients and renal transplant patients receiving immunosuppressive therapy, to analyze the dose-response relationship between UVB radiation and specific immunosuppression.

The estimated ED50_{human, Lis, LST} can be compared to previously published data on the effects of UVB exposure on humans. Several studies indicate that a total dose of 5.8 kJ/m² UVB radiation is enough to suppress CHS responses in Caucasians as well as in African Americans (4,6,30). However, the UV source used in these studies, a high-pressure mercury lamp, had a different spectrum compared with the FS40 lamp, and the irradiance was measured from 290 to 320 nm. Thus, these data do not easily compare to our estimated value. Cooper et al. (38) showed that even a single suberythemal dose of UVB radiation could induce local suppression of CHS responses. Results from their study were also difficult to relate to the estimated ED50_{human, Lis, LST} because

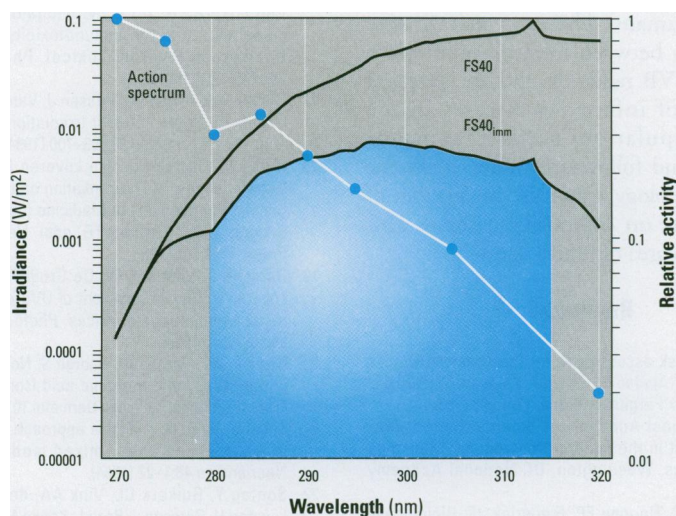


Figure 2. Method for the estimation of the biologically effective dose for immunosuppression of ultraviolet B radiation from the FS40 sunlamp (FS40_{imm}). The area below the FS40_{imm} curve was integrated between 280 and 315 nm. The formula for the calculation is

$$FS40_{imm} = \int d\lambda A(\lambda) \times \frac{dFS40(\lambda)}{d\lambda},$$

where FS40_{imm} = total biologically effective UVB dose, using the FS40 for UVB immunosuppression, $A(\lambda)$ = action spectrum for contact hypersensitivity as determined by De Fabo and Noonan (25), and $\frac{dFS40(\lambda)}{d\lambda}$ = measured UVB flux in W/m²/nm.

of dissimilarities in the exposure protocol and dosimetry of the experiments. Effects of *in vivo* UVB radiation on the MSLR activity of skin cells (39) indicate that cumulative doses of UVB radiation between 5 and 30 kJ/m² are needed to obtain suppression of the MSLR response. These values can be compared in some extent to the estimated ED50_{human, Lis, LST}, which is 13.1 kJ/m². Finally, Gilmour et al. (20) indicated that in psoriasis patients 30 kJ/m² UVB radiation could induce a suppression of the NK activity of peripheral blood mononuclear cells. It can therefore be concluded that the estimated ED50_{human, Lis, LST} is somewhere in the middle of the range of the published data on the effects of UVB exposure on humans.

Exposure assessment of UVB radiation must take into account the action spectrum used. UVB-induced immunosuppression has an action spectrum different from that of UVB-induced carcinogenesis. To estimate the biologically effective dose to which humans are exposed outdoors, the spectrum of the sun was multiplied by the action spectrum of contact hypersensitivity as determined by De Fabo et al. (3,25). It appears reasonable to suggest that an action spectrum for the UVB-induced effects on the immunological resistance to infectious diseases is similar to the action spectrum of UVB-induced immunosuppression of CHS because the effects on the resistance to infectious agents are often mediated by effects on specific T-cell responses. For example, in the immunological resistance to *L. monocytogenes* and *T. spiralis*, specific T-cell responses are important. In the CHS reaction, effects on specific T-cell responses are measured, and it is reasonable to postulate that these action spectra are comparable. It is, however, worthwhile to work out an action spectrum for the effects of UVB on the resistance to an infectious agent in rats. To assess possible risks due to UVB exposure for the incidence or severity of infectious diseases in a population, an action spectrum for increases in the load of *Listeria* bacteria in the spleen is more appropriate than just an action spectrum for the UVB-induced immunosuppression of specific T-cell responses to this pathogen.

Immune function studies (9,10) indicate that the immunological effects observed after UVB exposure daily for 14 consecutive days to 750 J/m² are not comparable to effects observed after daily UVB exposure for 7 days to 1500 J/m², in spite of equal total UVB doses. Thus, a cumulative dose of UVB radiation does not give enough information on the UVB-induced effects on the immune system; exposure intensity and exposure duration may play a role in the effects measured. The UVB dose that induced suppression in the *Listeria* model

was presented as a cumulative dose; however, in our experiments, the rats obtained this dose over a period of 7 days. Thus, it might be appropriate to divide the biological effective dose (BED_{human, Lis, LST}) by 7 and present that value as the daily UVB exposure for 7 consecutive days that could induce 50% immunosuppression of the specific T-cell response to *Listeria*. Thus, the total period necessary to induce 50% suppression of *Listeria*-specific T-cell response in July at 40°N would be less than 15 min daily for 7 consecutive days.

Risk assessment of the carcinogenic potential of UVB radiation is easier to determine than risk assessment of UVB-induced suppression of the immunological resistance to infectious diseases. For risk assessment of the carcinogenic potential of UVB radiation, it is relatively easy to quantify the adverse endpoint, and its adversity is beyond dispute. Decreases in T cell-specific immune responses to *Listeria* are difficult to quantify in terms of increases in incidence or severity of infectious diseases in a population. An increased infection load, leading to clinical effects, can be defined more definitely as an adverse effect and may be used as an endpoint for risk assessment.

In conclusion, although some infections such as *Schistosoma mansoni* (28) are not affected by UVB exposure, UVB exposure can impair the immunological resistance to many infectious diseases in rats (13–16). These data appear to be relevant for the human situation because many immunosuppressive effects found in animals can also be found in humans. The exact quantitative relationship between immunosuppressive effects of UVB radiation and an increased incidence of infectious diseases in the human population needs a cautious approach, and follow-up studies are essential. Epidemiology may help the analyses of UVB effects on infectious diseases and should be an area of future research.

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